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Gadolinium-Loaded Viral Capsids as Magnetic Resonance Imaging Contrast Agents

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Abstract

Polymeric nanohybrid P22 virus capsids were used as templates for high density Gd^{3+} loading to explore magnetic field-dependent (0.5–7.0 T) proton relaxivity. The field-dependence of relaxivity by the spatially constrained Gd^{3+} in the capsids was similar when either the loading of the capsids or the concentration of capsids was varied. The ionic longitudinal relaxivity, r_1 , decreased from 25–32 $\text{mM}^{-1} \text{s}^{-1}$ at 0.5 T to 6–10 $\text{mM}^{-1} \text{s}^{-1}$ at 7 T. The ionic transverse relaxivity, r_2 , increased from 28–37 $\text{mM}^{-1} \text{s}^{-1}$ at 0.5 T to 39–50 $\text{mM}^{-1} \text{s}^{-1}$ at 7 T. The r_2/r_1 ratio increased linearly with increasing magnetic field from about 1 at 0.5 T, which is typical of T_1 contrast agents, to 5–8 at 7 T, which is approaching the ratios for T_2 contrast agents. Increases in electron paramagnetic resonance line widths at 80 and 150 K and higher microwave powers required for signal saturation indicate enhanced Gd^{3+} electron spin relaxation rates for the Gd^{3+} -loaded capsids than for low concentration Gd^{3+} . The largest r_2/r_1 at 7 T was for the highest cage loading, which suggests that Gd^{3+} – Gd^{3+} interactions within the capsid enhance r_2 more than r_1 .

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Recent reviews emphasize the need for new types of contrast agents (CAs) for magnetic resonance imaging (MRI) at magnetic fields higher than those for which discrete Gd^{3+} complexes have been optimized [1–5]. MRI instruments in both clinical and research laboratories use a variety of magnetic fields (0.5–17.2 T) with an increasing trend towards higher fields [6–8]. Higher fields offer greater signal-to-noise ratio, improved spatial resolution, and reduced acquisition times, which have clear benefits for many applications including functional brain imaging and spectroscopy. However, at high magnetic fields, the relaxivity of some Gd^{3+} T_1 CAs is diminished relative to that at 1.5 T. Moreover, increased dosage to maintain the same effective signal contrast may introduce induced susceptibility effects. T_2^* susceptibility effects are well-known for bolus injections of administered Gd^{3+} CAs and can be problematic for quantitative clinical T_1 imaging [6, 9].

Small, fast tumbling molecules, such as gadolinium diethylenetriaminepentaacetate ($\text{Gd}(\text{DTPA})^{2-}$), exhibit longitudinal, r_1 , and transverse, r_2 , relaxivities that are approximately independent of applied magnetic field (B_0) strength, Figs. 2, 3. Typical strategies for improving r_1 relaxivity at about 1 T involve covalent attachment to macromolecular structures to increase rotational correlation times, τ_R . Attachment of Gd^{3+} to large macromolecules results in r_1 relaxivities that peak in the magnetic field range of 0.5–1.5 T and then sharply decrease with increasing field [10]. Thus, the strategy of slowing tumbling is less effective at higher magnetic fields.

Engineering CAs based on their r_2 relaxivities is an attractive alternative method for improved imaging agent performance at high magnetic field strengths where r_1 relaxivity decreases. Whereas T_1 CAs lose efficacy at high magnetic field strengths, T_2 CAs show improved contrast properties at higher clinical magnetic field strengths [11, 12], resulting in an increase of the r_2/r_1 ratio with increasing field.

Biocompatible nanostructures that have been used to enhance contrast properties include dendrimers, liposomes, polymers, proteins, nanoparticles, and protein cages, with a focus on chemical conjugations and encapsulations [10, 13]. Several protein architectures, including ferritins, heat shock proteins, and viruses, have been engineered to confer new MRI functionality by mutagenesis and chemical modifications [14–17]. Protein cages have well-defined monodisperse architectures with multivalent presentations of functional groups, which offer a distinct spatial distribution of amino acid side chains for further synthetic processing [18]. Previous studies have demonstrated the utility of the cage interior and exterior surfaces for covalent and noncovalent attachment of paramagnetic moieties for relaxivity enhancement [14–17, 19].

Bacteriophage P22 virus-like particle (VLP) is a protein cage architecture that has been engineered for functional hybrid nanostructures with various MRI proton relaxivity properties [15–17, 20]. Recently, polymeric nanocomposites within P22 protein cages have been synthesized to significantly increase the payload of CAs by utilizing the full interior three-dimensional capsid space [15]. The highly versatile P22 viral capsid was used previously for site-selective polymerization to form encapsulated polymer constrained nanocomposites of 2-aminoethyl methacrylate (AEMA) by atom-transfer radical polymerization (ATRP), Fig. 1. $\text{Gd}(\text{DTPA})^{2-}$ was covalently attached to the AEMA polymer

network with isothiocyanate linkers to form CAs with up to 9,100 Gd³⁺/cage [15]. Polymeric P22-Gd³⁺ constructs showed enhanced ionic relaxivity at 0.1–1.4 T and a significantly larger overall particle relaxivity than for a P22-Gd³⁺ nanocomposite comprising shorter oligomers [16]. P22-Mn³⁺ protoporphyrin IX samples were recently reported with loading-dependent r_2 and r_1 relaxivity, which demonstrated synthetic control over NMR relaxation properties [17]. Highly loaded Gd³⁺ and Mn³⁺ P22 particles revealed magnetic field-dependent r_1 and r_2 relaxivities that were determined by the paramagnetic species, spatial confinement, and loading factors. A general understanding of Gd³⁺ spatial confinement and magnetic field-dependent r_1 and r_2 is required to further design enhanced macromolecular CAs [21].

This work extends our earlier polymeric P22-Gd³⁺ studies to examine the Gd³⁺ loading dependence of r_1 and r_2 over a wider range of magnetic field strengths [15,17]. The experiments compare relaxivity at clinical MRI field strengths and beyond [22] for similar global Gd³⁺ concentration obtained with different combinations of cage loading and cage concentrations. Three sets of P22-Gd³⁺ samples were prepared in carbonate buffer at pH 9 for NMRD relaxivity experiments: (a) variable loading ranging from 1,730 to 5,500 Gd³⁺/cage with a constant cage concentration of 102 nM, (b) a constant loading factor of 7,200 Gd³⁺/cage with cage concentrations of 100, 75, 50, and 25 nM, and (c) a constant loading factor of 10,300 Gd³⁺/cage with cage concentrations of about 23, 17, 11, and 5.8 nM. The Gd³⁺ loading factors of P22 cages, Gd³⁺ concentrations inside the P22 cages, the global Gd³⁺ concentrations, and P22 cage concentrations for stock solutions are listed in Table S1. The concentrations of P22-Gd³⁺ samples and a comparison set of Gd(DTPA)²⁻ samples that were used in the NMRD experiments are shown in Table S2.

Relaxivity measurements for polymeric P22-Gd³⁺ were recorded at magnetic field strengths between 0.49 and 7.0 T. Relaxivities are calculated per Gd³⁺ ion. For all three P22-Gd³⁺ samples, the r_1 relaxivities were largest at 0.49 T (21 MHz), Fig. 2. Maximum r_1 relaxivity values were 25 mM⁻¹s⁻¹ (1,730–5,500 Gd³⁺/cage), 30 mM⁻¹s⁻¹ (7,200 Gd³⁺/cage), and 32 mM⁻¹s⁻¹ (10,300 Gd³⁺/cage), where r_1 relaxivity shows modest increases with cage loading. These relaxivities are comparable to previously reported values of 22 mM⁻¹s⁻¹ for branched oligomer P22-Gd³⁺ (1,900 Gd³⁺/cage, 0.65 T) and polymeric P22-Gd³⁺ (9,100 Gd³⁺/cage, 1.4 T) [15, 16]. For higher magnetic field strengths, the r_1 relaxivity decreases towards that of the unattached Gd(DTPA)²⁻ chelate.

The r_2 relaxivities for the P22-Gd³⁺ samples increased modestly as a function of magnetic field strength above 1.0 T, Fig. 3. The r_2 relaxivities ranged from 28 to 39 mM⁻¹s⁻¹ (1,730–5,500 Gd³⁺/cage), 32 to 47 mM⁻¹s⁻¹ (7,200 Gd³⁺/cage), 37 to 50 mM⁻¹s⁻¹ (10,300 Gd³⁺/cage), as a function of magnetic field strength, again showing higher r_2 relaxivities for increased cage loadings. The r_2 relaxivities were comparable to literature values of 27 mM⁻¹s⁻¹ for branched oligomer P22-Gd³⁺ samples [16] at 0.6 T, and values ranging from 2 to 47 mM⁻¹s⁻¹ for dendrimer conjugated lanthanide contrast agents [12] at 1.4 T.

The highest loaded sample (10,300 Gd³⁺/cage) exhibited the strongest T_1 relaxivity at low fields (Fig. 2) and the strongest T_2 relaxivity at high fields (Fig. 3). The ratios between longitudinal and transverse (r_2/r_1) relaxivities reflect the efficacy as T_1 or T_2 CAs. The r_2/r_1

ratios for the P22-Gd³⁺ and Gd(DTPA)²⁻ samples as a function of magnetic field are shown in Fig. 4. At all fields studied, r_1/r_2 for Gd(DTPA)²⁻ is about 1, which is characteristic of T_1 CAs. The r_2/r_1 for P22-Gd³⁺ at 0.49 T also is about 1, but increases with increasing magnetic field. MRI contrast agents with r_2/r_1 values >10.0, including superparamagnetic iron oxide nanoparticles, are used for T_2 -weighted imaging. The values of r_2/r_1 for P22-Gd³⁺ at high field approach those for T_2 CAs.

Gueron [23] pointed out that solvent relaxation by paramagnetic species can include a contribution, called the Curie effect, that is dependent on the total magnetization of the system, and independent of metal relaxation rate. Koenig [22] applied similar magnetic susceptibility concepts to MRI contrast agents. The trend for the P22-Gd³⁺ capsids from usual T_1 CA behavior at 0.49 T to T_2 CA values at 7 T suggests susceptibility effects from spatially confined Gd³⁺ ions. However, the susceptibility effects were significantly weaker than for typical iron oxide superparamagnetic nanoparticles [24]. Nanoparticle susceptibility effects are attributed to T_2 outer sphere relaxation of superparamagnetic agents ($r_2/r_1 > 10$). T_2 relaxation within the P22 cage is a type of susceptibility effect that occurs from high concentrations of Gd³⁺ ions, reminiscent of T_2^* effects from bolus injections of Gd³⁺ CA.

Electron paramagnetic resonance (EPR) spectroscopy was used to further understand the intra-particle molecular interactions that might contribute to relaxivity. Continuous wave (CW) EPR spectra of P22-Gd³⁺ at 80 and 150 K and 35 GHz (1.2 mT) exhibit $m_s = \pm 1/2$ transitions that are characteristic of $S = 7/2$ (Fig. S5). The line widths for the $m_s = \pm 1/2$ transitions increase significantly as the number of Gd³⁺ per cage is increased (Table S3), consistent with strong dipole–dipole interactions at the high local concentrations inside the cage. The line broadening indicates large decreases in T_{2e} . Power saturation curves support the conclusion that the electron spin relaxation rates for P22-Gd³⁺ are substantially faster than for low concentrations of Gd(DTPA)²⁻ (Table S4). The relaxation times for P22-Gd³⁺ at 80 K are too short to measure by pulsed EPR. Values of T_{1e} and T_{2e} for Gd³⁺ and Gd(DTPA)²⁻ at 80 K and 35 GHz obtained by pulsed EPR are about 1 μ s for concentrations in the range of 0.1–1 mM, but decrease rapidly at higher concentrations (Table S5). These measurements at low temperature suggest that Gd³⁺–Gd³⁺ interactions may also lead to enhanced electron spin relaxation at higher temperatures and magnetic fields [25]. The enhancement of Gd³⁺ relaxation rates arising from spin–spin interactions within the cage may contribute to increased r_2 proton relaxivity of the CAs at higher loading. The assumption that T_{1e} and T_{2e} are longer than tumbling correlation times may not be valid for P22 samples [2], especially at high loadings within macromolecular structures.

The use of supramolecular architectures is becoming a more common strategy to enhance CA relaxivities. Combinatorial approaches have attracted considerable interest for optimizing CA relaxivities that contain a large number of paramagnetic relaxation agents [14, 21, 26]. In particular, Chen et al. recently synthesized a library of polymeric Gd³⁺ DOTA supramolecular assemblies with many Gd³⁺ ions inside of nanoparticles to determine the ideal paramagnetic loading conditions for highest r_1 relaxivity [21]. In our system, the Gd³⁺ relaxivity mechanism exhibits T_1 -enhanced relaxivity at low magnetic fields and T_2 -enhanced relaxivity at high magnetic fields. The data underscore the importance of Gd³⁺ loading factors within macromolecular structures and protein cage dilutions with constant

paramagnetic concentrations on proton relaxivity properties. The experimental design can provide insight into quantitative MRI analysis of distinct Gd^{3+} spatial configurations and concentrations.

This work demonstrates that P22- Gd^{3+} relaxivity is similar to T_1 CAs at low fields and more like T_2 -enhanced CAs at high magnetic fields. The relaxation rates and relaxivity effect of the Gd^{3+} -loaded P22 capsids suggest greater importance of the Curie effect for these particles than for discrete Gd^{3+} complex of the type now used for low-field clinical MRI. These results suggest approaches to further design, engineer, and tune CA properties by chemical synthesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Schematic of polymeric P22 particles with spatially confined Gd^{3+} ions. The exterior diameter is 64 nm and the interior diameter is 54 nm. $\text{Gd}(\text{DTPA})^{2-}$ was covalently attached to the internal polymer scaffold with loading factors of 1,730–10,300 $\text{Gd}^{3+}/\text{cage}$

Fig. 2.

Comparison of r_1 NMRD curves for three P22-Gd³⁺ samples and Gd(DTPA)²⁻. Relaxivities were calculated from the slopes of plots of $1/T_1$ vs global Gd³⁺ concentration. The designation ‘variable loading’ refers to the series of samples in which the global Gd³⁺ concentration was varied by changing the loading of gadolinium in the cage, at constant cage concentration

Fig. 3. Comparison of r_2 NMRD curves for three P22-Gd³⁺ samples and Gd(DTPA)²⁻. Relaxivities were calculated from the slopes of plots of $1/T_2$ vs global Gd³⁺ concentration

Fig. 4.

Longitudinal and transverse relaxivity ratios (r_2/r_1) for each P22-Gd³⁺ configurations, P22-Gd³⁺ 10,300 Gd/cage (*filled circle*), P22-Gd³⁺ 7200 Gd/cage (*filled diamond*), P22-Gd³⁺ variable loading (*filled square*), Gd(DTPA)²⁻ (*filled triangle*). Relaxivity ratios for the P22-Gd³⁺ samples are intermediate between typical T_1 and T_2 CAs